Dosage Requirements of *Ultrabithorax* and *bithoraxoid* in the Determination of Segment Identity in *Drosophila melanogaster*

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ABSTRACT

The wild-type *Ultrabithorax* (*Ubx*) and *bithoraxoid* (*bxd*) functions are primarily responsible for establishing the identity of parasegment 6 (PS6) in the Drosophila embryo and thus the identity of the posterior compartment of the third thoracic segment (pT3) and the anterior compartment of the first abdominal segment (aA1) in the adult. The experiments described were designed to test the ability of an increased dosage of Ubx^+ and bxd^+ to affect the transformation of PS5 toward PS6. The results are consistent with the ideas that (1) multiple copies of Ubx^+ and bxd^+ cause some cells within PS5 to take on the characteristics of PS6 cells but do not cause an overall parasegmental transformation of PS5 toward PS6, (2) cellular identity depends not only on the activity of Ubx^+ but on its concentration as well, and (3) that an interaction between Ubx^+ and the wild-type *Antennapedia* (*Antp*) gene establishes segmental identity in pT2. In the first instar larvae carrying eight copies of Ubx^+ and bxd^+ the fine hairs of the T3 setal belt are transformed toward the hook-like structures of the A1 setal belt. Other structures within this segment are unaffected. In the adult, the haltere is reduced in size. The transformation of pT2 cells (wing) toward pT3 cells (haltere) is seen in adults carrying eight doses of wild type *Ubx* and *bxd* by decreasing the amount of the bithorax complex (BX-C) regulator *Polycomb* (*Pc*). However, the transformation of the T3 setal belt is not enhanced in the larvae of these animals. The interaction between the genes of the *Antennapedia complex* (*ANT-C*) and the Ubx^+ and bxd^+ functions in pT2 is dosage sensitive only when the animals carry one copy of *Pc*. In these animals, the transformation of wing toward haltere is significantly enhanced.

THE segmentation pattern of *Drosophila melanogaster* is generated by a hierarchical series of pattern forming events. A large number of genes, identified by mutations that disrupt various aspects of development, are responsible for this process (for an excellent review, see AKAM 1987). To summarize, maternal genes establish anterior/posterior gradients within the egg. The maternal and zygotic gap genes are activated at different positions along the anterior-posterior axis and establish large spatial domains within this embryo. The pair rule genes interact with the gap genes and are required to further subdivide the embryo into 14 separate parasegments. The genes of two major complexes, the *Antennapedia complex* (*ANT-C*) and *bithorax complex* (*BX-C*) are responsible for conferring specific identities to each parasegment. By the end of embryogenesis these parasegments have developed into the head, thoracic and abdominal segments of the first instar larva such that parasegment 5 (PS5) corresponds to the posterior compartment of the second thoracic segment (pT2) and the anterior compartment of the third thoracic segment (aT3) and so on (MARTINEZ-ARIAS and LAWRENCE 1985).

The initiation and maintenance of segment identity by the *ANT-C* and *BX-C* are also the result of complex gene interactions. These homeotic genes interact with the segmentation genes to establish the parasegmental patterns of homeotic gene expression (WHITE and LEHMANN 1986; DUNCAN 1986; INGHAM and MARTINEZ-ARIAS 1986). Homeotic gene expression also requires the activity of repressors (*e.g.*, LEWIS 1978; STRUHL 1981a, 1983; DUNCAN 1982), repressive interactions among the homeotic genes themselves (HAFEN, LEVINE and GEHRING 1984; HARDING *et al.* 1985; STRUHL and WHITE 1985), and presumably, regulation by factors involved in establishing cell type, to maintain these patterns throughout development.

Evidence from the mutational studies has demonstrated that there is a polarity in the repressive interactions among the homeotic genes. In the extended germ band stage, the *ANT-C* gene *Antennapedia* (*Antp*) is primarily expressed in PS4 and PS5 (MARTINEZ-ARIAS 1986; CARROLL *et al.* 1986). The distribution of wild-type *Antp* products is expanded posteriorly in animals deficient for the *BX-C* genes suggesting that $Antp^+$ is suppressed by the *BX-C* genes in most of the cells of PS6–PS13 (HAFEN, LEVINE and GEHRING

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1984; CAROLL *et al.* 1986). Similarly, the pattern of Ubx^+ expression is affected by the *abd-A* and *Abd-B* mutations (WHITE and WILCOX 1985b; STRUHL and WHITE 1985). One important question to ask is how this suppression is effected; how do the homeotic genes interact to establish segment-specific patterns of expression. One way to obtain information about the nature of these interactions is to determine whether they are sensitive to changes in gene dosage. For example, both Ubx^+ and $Antp^+$ are expressed in PS5 (AKAM and MARTINEZ-ARIAS 1985). If establishment of the final expression patterns of Ubx^+ and $Antp^+$ products requires an interaction between these products, then one might anticipate that their patterns could be altered by changing the relative dosage of Ubx^+ with respect to $Antp^+$ and/or other regulators that act within PS5.

In this report I assess the effect of varying the doses of wild-type *Ubx* and its regulatory region, *bithoraxoid* (*bxd*), on the transformation of PS5 toward PS6. I find that the cells within PS5 respond differentially to changes in the dosage of Ubx^+ and bxd^+: certain structures within PS5 are transformed toward PS6 structures while other PS5 structures remain unaffected. When these eight-dose animals carry only one copy of the *BX-C* regulator *Polycomb* (*Pc*), more PS5 cells exhibit the characteristics of PS6 cells. This transformation is enhanced further if the animal also carries a single copy of the *ANT-C*. These observations support the view of PEIFER, KARSH and BENDER (1987) that segments are mosaics of different cell types that express different batteries of homeotic genes. They are also consistent with the idea that the repressive interactions among the homeotic genes may be more complex than previous mutational analyses suggest.

MATERIALS AND METHODS

Mutations and strains: Most of the balancers and mutations used in this study are described in LINDSLEY and GRELL (1968). The *Dp(2;3)P10* is an insertion of the region including *spineless* (*ss*), *Ubx*, *bxd* and part of the *abd-A* regions of the *BX-C* into 29C of chromosome *2* (LEWIS 1978; KARCH *et al.* 1985). *Dp(3;3)P5* is a tandem duplication of the entire *BX-C* at 89E1-2 (LEWIS 1978) and *Dp(1;3)68* is an insertion of the *BX-C* into the heterochromatic region of the *X* chromosome (LEWIS 1986). One of the *TM6B* balancers carries the mutation *Tubby* (*Tb*) that is described by AUERBACH (1943) and CRAYMER (1980). The other has the dominant mutation Dr^{Mcio}. The *Polycomb* allele Pc^3 is described by LEWIS (1980). The two overlapping and nonidentical deficiencies for the *Antennapedia complex* used in these studies are *Df(3R)Scr* [SINCLAIR as cited in KAUFMAN (1978) and LEWIS (1980)] and $Df(3R)Antp^{Ns+R17}$ (DUNCAN and KAUFMAN 1975). The region of overlap for these two deficiencies is small and includes the *ANT-C* genes from *Scr* to *Antp* (KAUFMAN, LEWIS and WAKIMOTO 1980). The wild-type strain used in these studies is Canton-S.

Culture conditions: Flies were cultured on a medium of LEWIS (1960) in half-pint milk bottles supplemented with yeast. All crosses were reared at 25° and approximately 80% humidity.

Mutagenesis procedure and generation of tandem duplications of *Dp(2;3)P10*: In order to generate additional copies of the Ubx^+ and bxd^+ functions I generated tandem duplications of *Dp(2;3)P10*. For each of six mutageneses, approximately 200 *T(2;3)P10/Dp(2;3)P10*; *Ubx* virgin females were irradiated with 4500 r of X-rays using a Westinghouse 50-kV dual Dermadex unit (dose rate 400 r/min, 50 kV, 20 mA, 0.5 mm Al filtration). These females were then crossed to *Dp(1;3)68*; *Cbx Ubx* gl^3 males and allowed to mate for 24 hr before the males were discarded. The females were transferred every 3 days for three transfers. Male progeny with non-duplicated *Dp(2;3)P10* chromosomes have the enlarged halteres characteristic of *Ubx/+* animals while those heterozygous for a tandem duplication of *Dp(2;3)P10* have wild-type halteres. A total of 47,478 male progeny were scored and 32 of them had wild-type halteres. Of these 32, 23 were either sterile or died before mating. The remaining nine were fertile and the duplication chromosomes were balanced and kept in stock with *CyO* and *Cbx Ubx* gl^3. Two of the duplications, *DpP10(5)* and *DpP10(2)*, are cytologically indistinguishable from *Dp(2;3)P10*. One of the duplications, *DpP10(7)*, includes small portions of the material at 29C. The remaining duplications were either too large or were lost before they could be analyzed. The three small duplications were also put in stock with *CyO* and a duplication of the entire *BX-C*, *Dp(3;3)P5* balanced with *TM6B,Hu Tb*. Males and females from these stocks were crossed *inter se* to produce animals with eight doses of the Ubx^+ and bxd^+ functions.

Preparation and analysis of wings: *DpP10(5)*; Pc^3 *Dp(3;3)P5/Dp(3;3)P5* adults were generated by crossing *DpP10(5)*; Pc^3 *Dp(3;3)P5/TM6B,Hu Tb* females to *DpP10(5)*; *Dp(3;3)P5* males. Adults from the reciprocal cross were also analyzed. Pc^3 *Dp(3;3)P5/Dp(3;3)P5* adults were generated by crossing Pc^3 *Dp(3;3)P5/TM6B, Hu Tb* females to *Dp(3;3)P5/TM6B,Hu Tb* males. Again, the adults produced from the reciprocal cross were also analyzed. *DpP10(5)*; Pc^3 *Dp(3;3)P5/Df(3R)Scr* p^p and *DpP10(5)*; Pc^3 *Dp(3;3)P5/Df(3R)* $Antp^{Ns+R17}$ animals were constructed by mating *DpP10(5)/CyO*; Pc^3 *Dp(3;3)P5/TM6B,Hu Tb* females to *DpP10(5)/CyO*; *Df(3R)Scr* p^p*/TM6B, Hu* Dr^{Mcio} or *DpP10(5)/CyO*; *Df(3R)*$Antp^{Ns+R17}$*/TM3* males. Controls for the *Dp(3;3)P5* chromosome were constructed by crossing *DpP10(5)*; Pc^3 *Dp(3;3)P5/TM6B,Hu Tb* females to either Canton-S or *DpP10(5)*; $p^p e$*/TM6B, Hu* Dr^{Mcio} males. Control animals were collected from a Canton S stock. The adult progeny were kept in a preserving solution of 1 part glycerol:3 parts 95% ethanol (v:v) for at least 24 hr. The wings were dissected away from the bodies, placed in isopropanol for a few minutes and mounted in a solution of 2 g/ml of Canada balsam in methyl salicylate (STRUHL 1981b). The slides were weighted and left on a slide warmer set at 60° for 2–3 days. All preparations were viewed under a compound microscope.

First instar larvae: Larvae homozygous and heterozygous for the three duplications of *Dp(2;3)P10* were isolated from *inter se* crosses among *Dp(3;3)P5/TM6B,Hu Tb* parents that were also homozygous for *DpP10(5)*, *DpP10(2)* or *DpP10(7)*. Larvae from these crosses and the reciprocal crosses were analyzed. Homozygous *Dp(3;3)P5* larvae were isolated from a *Dp(3;3)P5/TM6B,Hu Tb* stock. The *DpP10(5)*; Pc^3 *Dp(3;3)P5/Dp(3;3)P5* animals were obtained from a cross of *DpP10(5)*; Pc^3 *Dp(3;3)P5/TM6,Hu Tb* females to *DpP10(5)*; *Dp(3;3)P5/TM6B,Hu Tb* males. The Pc^3 *Dp(3;3)P5/Dp(3;3)P5* larvae were obtained from a mating of Pc^3 *Dp(3;3)P5/TM6B,Hu Tb* females to *Dp(3;3)P5/*

TM6B,Hu Tb males. Larvae collected from the reciprocal crosses were also analyzed. The wild-type controls were isolated from a Canton-S stock. Parents were mated for 2–3 days and placed in split bottles of regular food supplemented with yeast for 24 hr at 25°. The eggs were collected, washed in distilled water and allowed to develop overnight in a Petri dish of distilled water. The first instar larvae were scored for Tb^+ (the tracheal trunks of *Tb* homozygotes or heterozygotes are more coiled than those of the Tb^+ animals) and mounted in a 9:1 lactic acid:95% ethanol solution (LEWIS 1978). The slides were cleared for 16–24 hr on a slide warmer set at 51° and examined by phase microscopy the next day.

Statistics: The transformation of wing tissue to haltere tissue caused by mutations in *Pc* is highly variable. $Pc^3/+$ individuals differ in wing morphology as do the wings of any given $Pc^3/+$ heterozygote. Therefore, I have used the number of haltere specific structures (the sensilla trichodea) on the wing as a simple quantitative measure of transformation. These sensilla were counted and the means (Y) and standard deviations (s) determined. I tested the significance of differences between the variances and as the variances among the heterozygous Pc^3 populations were shown to be equal, I used a t-test to determine the significance of differences among the sample means.

RESULTS

Analysis of animals carrying multiple copies of the *Ubx/bxd* region: Animals homozygous for the tandem duplication of the entire *BX-C*, *Dp(3;3)P5*, and heterozygous for each pair-wise combination of the *Dp(2;3)P10* tandem duplications generated during this study, were used to determine the phenotype conferred by eight doses of the Ubx^+ and bxd^+ functions. Use of the *trans* heterozygotes rules out the possibility that additional material at 29C is responsible for the phenotype while a comparison of the eight-dose animals to those homozygous for the same *Dp(3;3)P5* chromosome eliminates effects due to the *Dp(3;3)P5* breakpoints and the extra doses of the abdominal region of the *BX-C*.

Both adult and larval phenotypes are identical in *DpP10(5)*; *Dp(3;3)P5,DpP10(2)*; *Dp(3;3)P5,DpP10(7)*; *Dp(3;3)P5,DpP10(5)/DpP10(2)*; *Dp(3;3)P5, DpP10(5)/DpP10(7)*; *Dp(3;3)P5*, and *DpP10(2)/DpP10(7)*; *Dp(3;3)P5* animals. The *DpP10(2)* and *DpP10(7)* homozygotes are extremely weak, so the following dosage studies were performed using *DpP10(5)*.

Animals carrying eight doses of the *Ubx/bxd* region show discrete but weak transformations and changes in phenotype suggesting that the extra doses of these functions are appropriately regulated. The adults are often missing the T3 haltere or have halteres that are greatly reduced in size. Otherwise these animals are completely wild type. While mutations in the bxd^+ or Ubx^+ transcription units can cause a transformation of haltere tissue to wing tissue, it appears that increased amounts of the Ubx^+ and bxd^+ functions can result in the reduction or loss of the haltere structure. An analysis of the first instar larvae carrying eight doses of the *Ubx/bxd* region suggests that this reduction may represent a transformation of T3 toward a more abdominal identity.

A comparison of wild-type (two-dose), *Dp(3;3)P5* (four-dose) and *DpP10(5); Dp(3;3)P5* (eight-dose) first instar larvae is pictured in Figure 1. In wild-type larvae, the ventral setal belts of the three thoracic segments are distinguished by differing bands of fine denticles. The T3 setal belt has a few denticles that are slightly thicker than the denticles found in T2. The three thoracic segments T1, T2 and T3 all contain bilaterally symmetric pairs of sensory organs, the Keilins organs and ventral pits. The first and second abdominal segments, A1 and A2 have broad ventral setal belts that are characterized by darkly pigmented hook-like hairs. In addition, A2 has an anterior row of anteriorly pointing hairs. SZABAD, SCHUPBACH and WIESCHAUS (1979) define the segment boundary by the major line of muscle attachment sites. This shifts the boundary more posteriorly and places the anterior row of A2 into the posterior compartment of A1. In homozygous *Dp(3;3)P5* larvae, the abdominal setal belts appear more dense and the A2 anterior row contains more hairs. In 2 of the 32 animals scored, at least one anteriorly directed hair appears anterior to the A1 setal belt. The two thoracic segments, T1 and T2 are wild type. The fine denticles of the T3 setal belt are darker than normal and a few small abdominal-like hooks begin to appear. In animals homozygous for *Dp(3;3)P5* and heterozygous or homozygous for the tandem duplications of *Dp(2;3)P10*, the T1 and T2 setal belts are wild type and the T3 setal belt is more abdominalized than it is in the *Dp(3;3)P5* homozygotes. Although the Keilins organs and ventral pits are still present and the shape of the setal belt remains essentially wild type, most of the denticles are now the hook-like hairs seen on the abdominal setal belts. Furthermore, 13 of the 39 animals scored have at least one and sometimes four anteriorly directed hairs anterior to the A1 setal belt. This difference is significant ($0.05 > P > 0.01$) and suggests that the extra doses of the Ubx^+ and bxd^+ functions are responsible for the expression of these hairs. However, as the four-dose and eight-dose animals could not be scored as siblings of the same mating, the variation in the number of anteriorly directed hairs could be due to differences in genetic background. The observation that 2 of 16 *DpP10(5)* homozygotes (which have six doses of the *Ubx/bxd* region and two doses of the abdominal region) carry anteriorly directed hairs anterior to the A1 setal belt argues against this possibility. For comparison, none of the 70 wild-type larvae analyzed have anteriorly directed hairs anterior to A1.

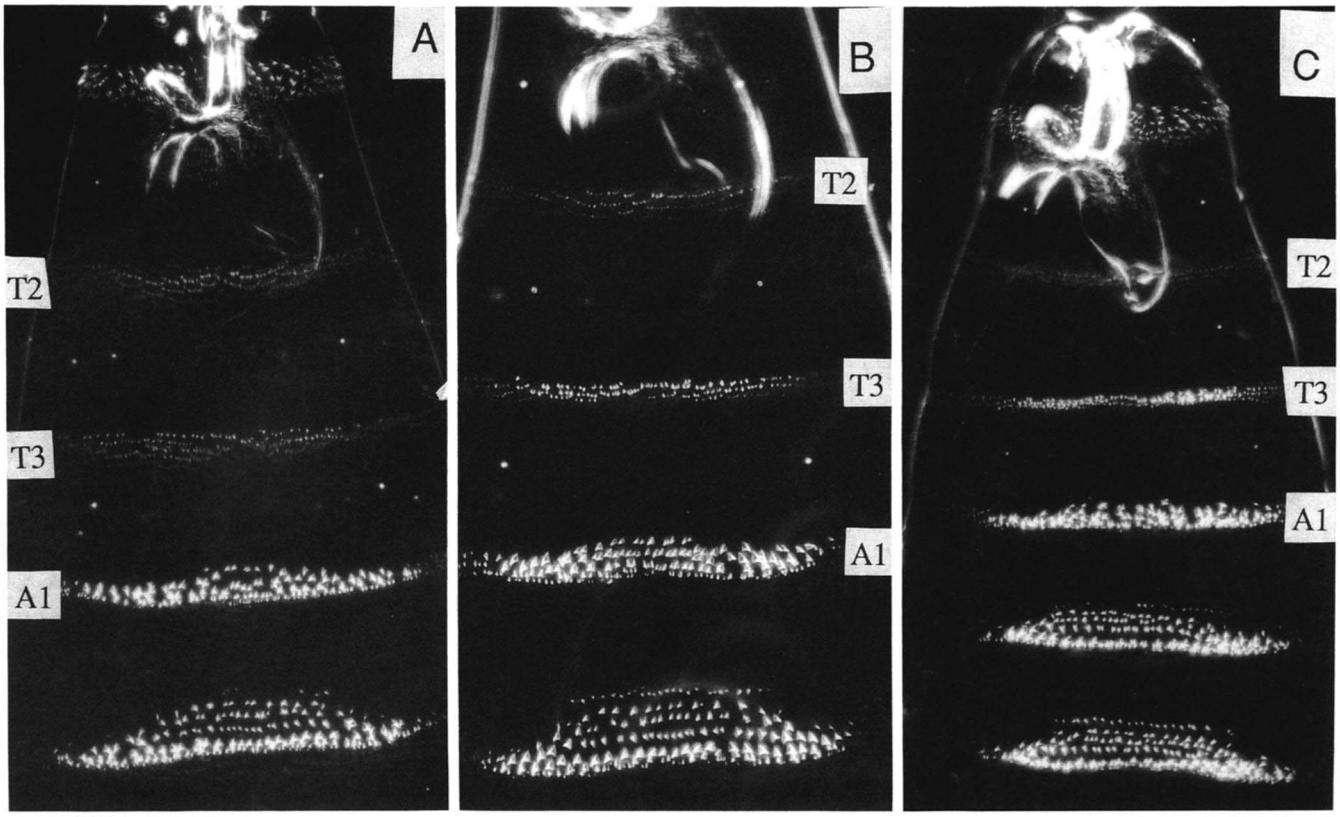

FIGURE 1.—(A) Ventral setal belts of a wild-type first instar larva (two doses of the Ubx^+ and bxd^+ functions). (B) Ventral setal belts of a first instar larva homozygous for *Dp(3;3)P5* (four doses of the Ubx^+ and bxd^+ functions). (C) Ventral setal belts of a first instar larva homozygous for *DpP10(5)* and *Dp(3;3)P5* (eight doses of the Ubx^+ and bxd^+ functions). Note that as the doses of Ubx^+ and bxd^+ increase, the setae of the T3 setal belt become hook-like and more refractile resembling the setae of the A1 setal belt. However, the size and shape of the T3 setal belt remains essentially wild type.

The effect of *Polycomb* on the phenotype of animals carrying multiple copies of the *Ubx/bxd* region: The results described above demonstrate that increasing the dosage of Ubx^+ and bxd^+ functions does not transform the entire T3 segment toward a more abdominal identity but does cause some T3 cells to exhibit a more abdominal identity. For example, the ventral pits are absolutely unaffected by the increased dosage of bxd^+ function, which is primarily responsible for suppressing the development of these organs in A1–A8 (LEWIS 1978). On the other hand, the increased amounts of Ubx^+ and bxd^+ functions cause the T3 setal belt to produce hook-like denticles and some of the posterior T3 cells to produce anteriorly directed hairs anterior to A1.

LEWIS (1978) first described *Pc* as a negative regulator of the *BX-C*. Animals homozygous for deficiencies of the *Pc* locus exhibit a transformation of all thoracic and abdominal setal belts towards the posterior-most A8 setal belt and have defects in head structures and involution. This transformation is not complete in the three thoracic segments: the Keilins organs and ventral pits are only partially suppressed and the ventral setal belts, though abdominalized, do not resemble that of A8. This suggests that in the absence of the wild-type *Pc* product, genes of the *BX-C* are no longer expressed differentially along the body axis but are expressed in a manner that approximates that in A8. The role of *Pc* as a negative regulator of the *BX-C* is supported by the observation of DUNCAN and LEWIS (1982) that four doses of the *BX-C* enhance this transformation. In these animals, all thoracic setal belts are characteristic of A8 and the ventral pits and Keilins organs are completely suppressed.

Given the role of Pc^+ as a regulator of *BX-C* function, I wanted to determine whether the abdominalization of T3 in animals carrying eight copies of the *Ubx/bxd* region could be enhanced by reducing the dose of the *Pc* locus. I reasoned that eight doses of Ubx^+ and bxd^+ might be sufficient to titrate the repressor expressed in a $Pc^3/+$ animal and affect a more complete transformation of T3 toward A1. This result was not obtained. The first instar larvae of *DpP10(5)*; Pc^3 *Dp(3;3)P5/Dp(3;3)P5* are identical to the *DpP10(5)*; *Dp(3;3)P5* animals pictured in Figure 1. The T3 setal belt is not enhanced, the Keilins organs and ventral pits are still present and the frequency of anteriorly directed hairs anterior to A1 remains the same. However, the adult phenotype demonstrates that the heterozygosity for the Pc^3 allele in these

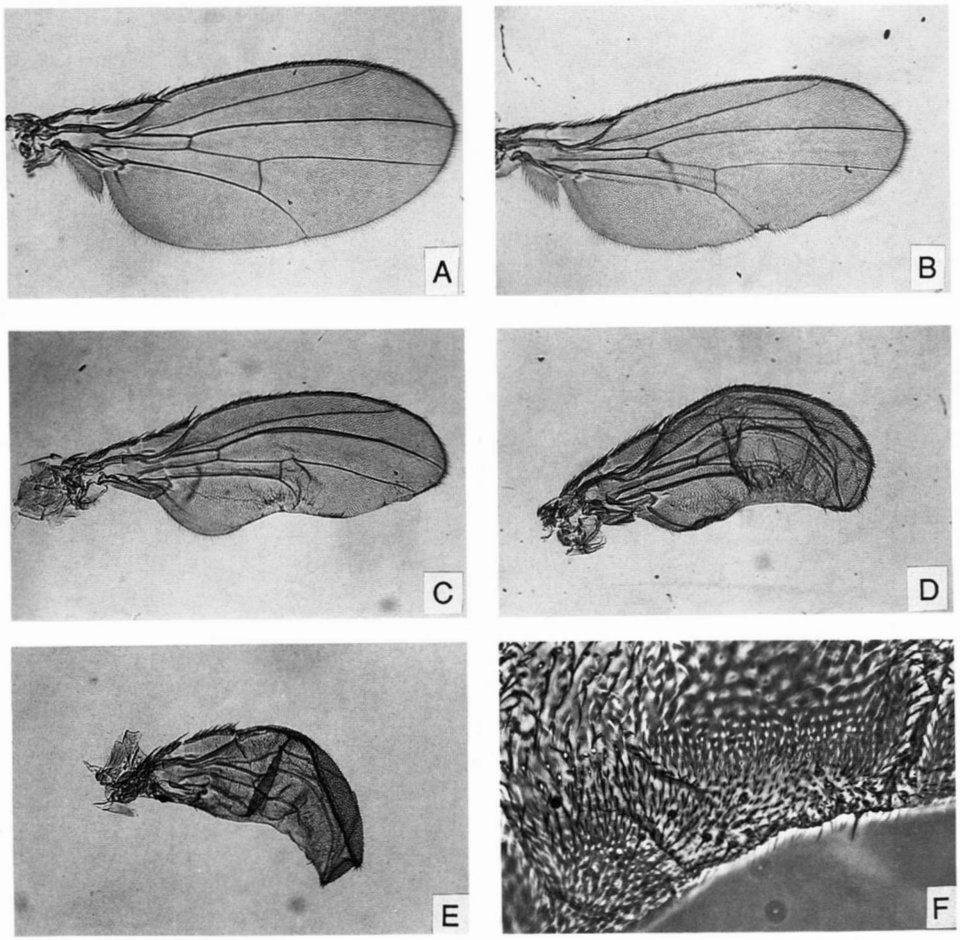

FIGURE 2.—(A) Wing from a wild-type adult. (B) This wing represents the most prevalent wing phenotype found in a population of Pc^3; $Dp(3;3)P5/Dp(3;3)P5$ animals (one copy of Pc^+ and four doses of the Ubx^+ and bxd^+ functions). (C) This wing represents the most prevalent wing phenotype found in $DpP10(5)$; Pc^3; $Dp(3;3)P5/Dp(3;3)P5$ animals (one copy of Pc^+ and eight doses of the Ubx^+ and bxd^+ functions). (D) This wing represents the most prevalent phenotype found in a population of $DpP10(5)$; Pc^3; $Dp(3;3)P5/Df(3R)$ $Antp^{NS+R17}$ animals (one copy of Pc^+, seven doses of the Ubx^+ and bxd^+ functions and one copy of the *ANT-C*). (E) This wing represents the most prevalent phenotype of the wings found among $DpP10(5)$; Pc^3; $Dp(3;3)P5/Df(3R)Scr$ animals (dosages as in D). All wings were photographed at the same magnification. (F) Higher magnification of a transformed wing showing the detail of an haltere patch and sensilla trichodea.

multiple dose animals does affect a transformation of some T2 cells into T3 cells. The extent of the transformation appears to be more extreme than that found in $Pc^3/+$ heterozygotes or $Pc^3/+$ heterozygotes with four copies of the *BX-C*. A comparison of the wings from these animals is presented in Figure 2. Although some T2 to T3 transformation has been seen in $Pc^3/+$ heterozygotes (LEWIS 1978) I observed only 2 of 38 wings that exhibited slight serration of the posterior wing margin. In no case could I find any evidence of haltere tissue in the wing. All of the wings from Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ animals have a serrated posterior wing margin and, in many, the veins L4 and L5 fail to reach the wing margin. Patches of haltere tissue, recognizable on the basis of pigmentation, tricome pattern and the presence of sensilla trichodea, often appear at that point where the L4 or L5 vein would ordinarily intersect the wing margin.

In Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ animals heterozygous or homozygous for $DpP10(5)$, the posterior wing margin is flattened and more serrated than that found in the Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ animals. The L4 and L5 veins fail to reach the posterior margin in most of the wings and many more of the wings have patches of haltere tissue. The phenotype of $Pc^3/+$ heterozygotes is extremely variable. The mild *Pc* effect observed in some of the wings from animals carrying six or eight doses of the *Ubx/bxd* region overlaps the phenotype of the wings from animals carrying four copies of the *Ubx/bxd* region. Because of this variability I used the sensilla trichodea as a quantitative measure of the wing to haltere transformation to determine the difference, if any, among the $Pc^3/+$ heterozygotes carrying four, six and eight doses of the *Ubx/bxd* region (Figure 3).

Most of the Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ wings (23/33) had no sensilla trichodea, four had one, four others had two and one wing each had three and six sensilla. The mean number of sensilla in this population is 0.64 ± 1.27. A much smaller fraction of the wings of the $Pc^3/+$ heterozygotes with six doses of the Ubx^+ and bxd^+ functions (10/36) exhibited no sensilla. The mean number of sensilla for this wing sample is 6.0 ± 6.14. The distribution of sensilla in the wings of $Pc^3/+$ heterozygotes with eight doses of the *Ubx/bxd* region is the same as that in the six-dose animals. Only 7 of the 47 wings scored have no sensilla and the mean number for this sample is 7.4 ± 6.4. The large standard deviations in all cases probably reflect the variability of the $Pc^3/+$ phenotype. The variances

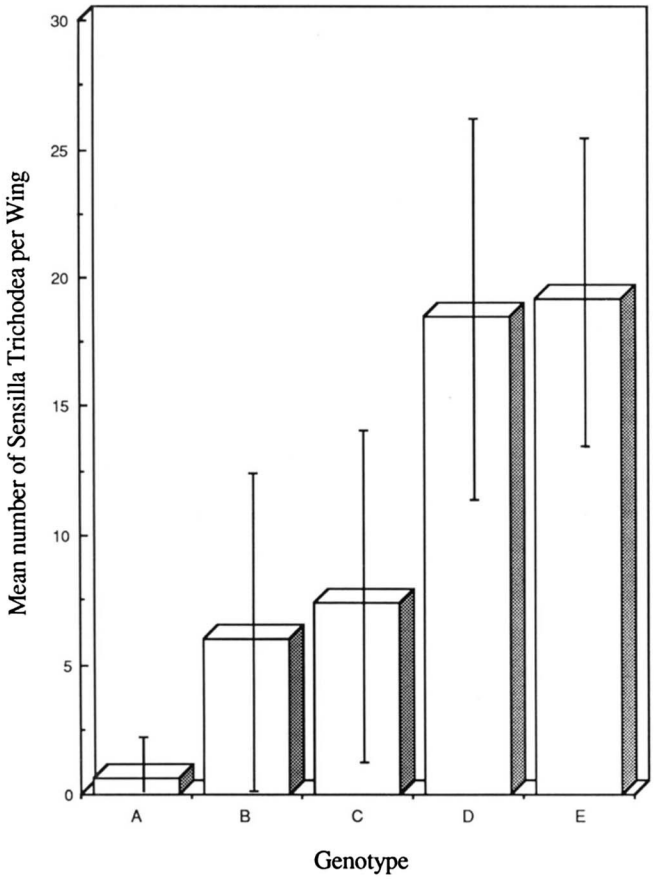

FIGURE 3.—(A) Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ (four doses of Ubx^+ and bxd^+). (B) $DpP10(5)/+$; Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ (six doses of Ubx^+ and bxd^+. (C) $DpP10(5)$; Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ (eight doses of Ubx^+ and bxd^+). (D) $DpP10(5)$; Pc^3 $Dp(3;3)P5/Df(3R)Scr$ (one copy of *ANT-C* and 7 doses of Ubx^+ and bxd^+). (E) $DpP10(5)$; Pc^3 $Dp(3;3)P5/Df(3R)Antp^{NS+R17}$ (same dosages as in D). The standard deviations are large because of the variability in the *Pc* phenotype however the means between the eight-dose animals and the eight-dose animals hemizygous for the *ANT-C* are significantly different. The means of the $Df(3R)Scr$ and $Df(3R)Antp^{NS+R17}$ strains are not significantly different nor are the means of the animals carrying six or eight copies of Ubx^+ and bxd^+.

of the two populations of $Pc^3/+$ heterozygotes carrying six and eight doses of the *Ubx/bxd* region are the same ($P \gg 0.2$) and the means are not significantly different ($P = 0.3$). The standard deviation of the Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ population was smaller and the variance significantly different from the other two populations ($P \ll 0.01$). A large standard deviation is not expected for this genotype as the wings are not much different from wild type and are not transformed to any great extent. A χ^2 analysis demonstrates that the number of transformed wings (those having at least one sensilla trichodea) in the Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ population is significantly different from the number of transformed wings in the other two populations ($P \ll 0.001$). As predicted, a χ^2 analysis of the six and eight dose $Pc^3/+$ heterozygotes shows that the number of transformed wings in the two populations is not significantly different ($0.1 < P < 0.2$).

To rule out the possibility that the $Dp(3;3)P5$ chromosome had accumulated modifiers that suppress the wing transformation, wings from $DpP10(5)/+$; Pc^3 $Dp(3;3)P5/+$ (five doses of Ubx^+ and bxd^+) and $DpP10(5)$; Pc^3 $Dp(3;3)P5/+$ (seven doses of Ubx^+ and bxd^+) control animals were also scored. The $DpP10(5)/+$; Pc^3 $Dp(3;3)P5/+$ wings were similar in phenotype to the Pc^3 $DP(3;3)P5/Dp(3;3)P5$ wings. The population had 26/46 wings with no sensilla, five wings with one sensilla, seven with two and three, four, five and six sensilla on each of two wings. The mean number of sensilla for this wing sample is 1.20 ± 1.75. The variance in this population is not significantly different from that found for the Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ ($0.10 > P > 0.05$) nor is the mean number of sensilla significantly different between the two populations ($0.2 > P > 0.1$). The variance of this wing population is significantly different from the variances of all other wing populations ($P \ll 0.01$). The $DpP10(5)$; Pc^3 $Dp(3;3)P5/+$ wing population has a distribution of sensilla similar to the $DpP10(5)/+$; Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ and $DpP10(5)$; Pc^3 $Dp(3;3)P5/Dp(3;3)P5$ wing populations. The mean number of sensilla for this population is 5.78 ± 5.84. The variance of this population is not significantly different from that of the $Pc^3/+$ heterozygotes carrying either six or eight copies of the *Ubx/bxd* region ($P > 0.20$). The mean number of sensilla is not significantly different from either the $Pc^3/+$ heterozygotes with six ($0.3 > P > 0.2$) or eight ($0.9 > P > 0.8$) copies of the *Ubx/bxd* region.

The effect of a single copy of both *Polycomb* and *ANT-C* on the phenotype of animals carrying multiple copies of the *Ubx* and *bxd* regions: The molecular analysis of AKAM and MARTINEZ-ARIAS (1985) demonstrated that at the extended germ band stage of development both Ubx^+ and $Antp^+$ are expressed in posterior T2/anterior T3 [*i.e.*, parasegment 5 (PS5)]. Those cells that do not express Ubx^+ products express $Antp^+$ products. Previous work has shown that in $Antp^-$ larvae, the denticles of the T3 setal belt are intermediate between those of T1 and A1 (WAKIMOTO and KAUFMAN 1981; STRUHL 1983; MARTINEZ-ARIAS, 1986). This suggests that the $Antp^+$ and Ubx^+ gene products may interact to affect the identity of PS5. To test the possibility that the two homeotic loci interact in a dosage sensitive manner, I constructed animals deficient for one copy of *ANT-C* and carrying multiple copies of the *Ubx/bxd* region.

In order to minimize genetic background effects, I used two overlapping but genetically unrelated deficiencies for the *ANT-C*: $Df(3R)Scr$ and $Df(3R)Antp^{Ns+R17}$. I reasoned that I could be confident of a dosage interaction if it could be demonstrated in

TABLE 1

Effect of *ANT-C* deficiencies on the transformation of wing to haltere in Pc^3 heterozygotes carrying multiple copies of the Ubx^+ and bxd^+ functions

Number of gene copies			
BX-C	Pc^+	*ANT-C*	Wing transformation
2	2	2	
4	2	2	
6	2	2	
8	2	2	
4	1	2	Weak
6	1	2	Intermediate
8	1	2	Intermediate
7	1	1	Strong
7	2	1	
5	2	1	
3	2	1	
2	2	1	

two different backgrounds. A summary of the results from these studies is presented in Table 1. Heterozygosity for either of the *ANT-C* deficiencies has no effect on the phenotype of animals with two, three, five or seven doses of the *Ubx/bxd* region. These animals are wild type except for the reduction in haltere size caused by the increased doses of the *Ubx/bxd* region and the dominant reduction of sex combs caused by a haploinsufficiency for the *Scr* gene in the *ANT-C*. However, this hemizygosity for *ANT-C* did enhance the transformation of wing to haltere in Pc^3/+ heterozygotes carrying seven doses of the Ubx^+ and bxd^+ functions. Wings from these animals are pictured in Figure 2 panels D and E. The overall wing size and posterior wing margin are reduced in these animals. The venation is also affected; L4 and L5 are shortened, L3 often fails to reach the wing blade and the posterior cross vein is frequently missing. All of the wings have a large patch of transformed tissue that often fills much of the reduced posterior compartment. The wings from the *Df(3R)Scr* heterozygotes have no alula while the alula from the wings of the $Df(3R)Antp^{Ns+R17}$ heterozygotes are reduced. As before, heterozygosity for Pc^3 causes a variability in the wing phenotype and the milder effects overlap the more extreme wing phenotypes from Pc^3/+ heterozygotes carrying six or eight copies of Ubx^+ and bxd^+. To determine the significance of the differences observed, the wings were dissected, mounted and the sensilla trichodea scored (Figure 3). The animals heterozygous for Pc^3/+ and *Df(3R)Scr*/+ and carrying seven doses of the *Ubx/bxd* region have a mean of 18.5 ± 7.4 sensilla per wing. Wings from the *DpP10(5)*; Pc^3 $Dp(3;3)P5/Df(3R)Antp^{Ns+R17}$ animals have a mean of 19.2 ± 6 sensilla. The variances of these populations are the same ($P > 0.2$) and their means are not significantly different ($0.7 < P < 0.8$). The means and standard deviations for all of the genotypes analyzed are presented in Figure 3. The statistical analysis shows the Pc^3/+ heterozygotes with six and eight doses are not significantly different in the extent of their transformation. Likewise, the *DpP10(5)*; Pc^3 *Dp(3;3)P5/Df(3R)ANT-C* animals are transformed to the same degree. However, the two sets of populations are significantly different from each other ($P \ll 0.001$) demonstrating that reducing the *ANT-C* copy number to one can affect the T2 transformation seen in Pc^3/+ heterozygotes with eight doses of the *Ubx/bxd* region.

Reducing the dose of Pc^+ in animals carrying eight doses of Ubx^+ and bxd^+ did not enhance the slight transformation of T3 observed in animals carrying only eight copies of the *Ubx/bxd* region. The *DpP10(5)*; Pc^3 *Dp(3;3)P5/Df(3R)Scr* and *DpP10(5)*; Pc^3 $Dp(3;3)P5/Df(3R)Antp^{Ns+R17}$ animals have halteres that are reduced and often missing but there is no loss of T3 legs or other indication that T3 is further abdominalized.

DISCUSSION

Much can be learned of gene action by an analysis of mutations. For example, we know from LEWIS' mutational analysis of the *BX-C* (1978) that the primary role of the wild-type *Ubx* and *bxd* functions is to determine the identities of the third thoracic and first abdominal segments. Mutations in *Ubx* cause the transformation of T3 structures towards those of T2 demonstrating that it normally suppresses T2 structures, such as the wing, in T3 and promotes the formation of T3 structures, such as the haltere. Larvae carrying mutations in the *bxd* region fail to suppress the ventral pits in the first through eighth abdominal segments. In the adult, *bxd* mutations cause the first abdominal segment to take on characteristics of the third thoracic segment. These results and those of an extensive mutational analysis of the *BX-C* led Lewis to propose that each function within the complex was responsible for the activation/repression of particular sets of structures, that each function was activated in the order in which it was expressed along the body axis and that once a particular function was activated it remained activated in all more posterior segments. According to the model, *Ubx* would be activated in the third thoracic and eight abdominal segments, connecting the tracheal trunk down the length of the larva and conferring a T3-specific identity to the T3 setal belt. In the adult, *Ubx* would suppress the formation of wing structures in all of the segments and induce the expression of haltere specific genes in T3. The next function, *bxd*, would be activated in the first abdominal segment and act to suppress the formation of ventral pits in A1 through A8 and promote A1 specific characteristics in A1. This pattern of activation/repression would be reiterated for each successive

function within the *BX-C* and the additive effects of each function would then define the identity of each segment.

The molecular analysis of *BX-C* mutant animals and animals carrying mutations in the regulators of the complex has added to the knowledge gained from the genetic analysis and modified some aspects of the model. While Ubx^+ is expressed in T3 through A8, it is not expressed equally in all cells in all segments; its primary site of expression is in PS6 (pT3/aA1), with weaker and varied patterns of expression in PS5 and PS7–13 (AKAM and MARTINEZ-ARIAS 1985; WHITE and WILCOX 1985a; BEACHY *et al.* 1985). In parasegments posterior to PS6, Ubx^+ is suppressed at least in part by $abd\text{-}A^+$ and $Abd\text{-}B^+$ functions (WHITE and WILCOX 1985b; STRUHL and WHITE 1985) and the *bxd* region acts to regulate Ubx^+ expression in PS6 and posteriorparasegments (BEACHY *et al.* 1985; WHITE and WILCOX 1985b; PEIFER, KARSH and BENDER 1987). Together, these results have led PEIFER, KARSH and BENDER (1987) to propose that segment identity is not strictly determined by the positive additive effects of sequentially activated *BX-C* functions, but by the differential patterning of homeotic gene expression within each parasegment. This mosaicism would be determined in *cis* by the large regulatory regions upstream from *Ubx*, *abd-A* and *Abd-B* and in *trans* through the interactions among these gene products.

In addition to the mutational analysis, a study of gene dosage effects is useful in determining the genetic interactions that establish and maintain the segmental patterns of homeotic gene expression. For example, can increased doses of the Ubx^+ gene or its product titrate Ubx^+ repressors in PS5 and cause this parasegment to take on the pattern of Ubx^+ expression characteristic of PS6? Similarly, if the homeotic genes interact in a manner that determines the homeotic gene expression in a particular cell, can the identity of a given cell be altered if the relative dosage of the homeotic genes is perturbed?

Not all cells within a segment are sensitive to increased doses of *Ubx* and *bxd*: The results presented above show that increasing the copy number of Ubx^+ and bxd^+ from two to eight is not sufficient to transform the identity of PS5 to PS6; the ventral pits and Keilins organs are not suppressed and the size and shape of the T3 setal belt remains wild type. This is not because the extra doses of *Ubx* and *bxd* are regulated to yield wild-type levels of expression. The *Ubx* protein, as assayed by *Ubx* antibody staining to embryos, is expressed in increasingly higher concentrations in the animals with four, six and eight copies of *Ubx* and *bxd* (WELCOME BENDER, personal communication). The sensitivity of the molecular analysis is enhanced by the phenotypic analysis. The antibody staining alone may not be sensitive enough to detect small increases in the concentration of *Ubx* protein. However, the fact that the T3 Keilins organs and ventral pits remain unaltered suggests that Ubx^+ expression in these cells remains at wild-type levels or, if increased, is not brought up to the levels found in the cells overexpressing *Ubx* protein. In either case, the pattern of Ubx^+ expression is not significantly altered in animals carrying multiple copies of the *Ubx/bxd* region. Interpreted within the context of the mosaic model of segmentation, these results imply that the copy number of *bxd*, the *cis*-regulatory region that determines the PS6 pattern of Ubx^+ expression, is not sufficient to titrate its PS5 repressors. However, the identity of specific PS5 cells, presumably those that normally express Ubx^+ and bxd^+ at some level, does change in response to the increased levels of these functions; as the dose increases, the T3 setae change from fine hairs to more A1-like hooks. One interpretation of this result is that increasing the level of Ubx^+ product in PS5 toward the levels found in the homologous PS6 cells causes a transformation of these PS5 cells towards a PS6 identity. Alternatively, it may be that in wild-type animals, the Ubx^+ product promotes denticle size and the cells of the A1 setal belt that express higher levels of Ubx^+ than the homologous cells in T3 are simply exaggerating the phenotype of the wild-type T3 denticles. It is expected that in this case, increasing the Ubx^+ levels in these T3 cells with multiple copies of the *Ubx/bxd* region would result in the A1 "transformation." Similarly, the reduction of the haltere observed in animals with increased dosages of the Ubx^+ product would suggest that increased levels of Ubx^+ in T3 cells that normally express lower levels cause these cells to repress the development of haltere structures as the homologous cells would in A1. Whether the A1 cells that express Ubx^+ have an exaggeration T3 phenotype or the T3 transformation toward A1 observed in animals with increased levels of Ubx^+ represents a transformation of cell type, these results suggest that segmental identity depends on the pattern of cells expressing Ubx^+ and on the levels of Ubx^+ product in each cell. While the haltere-to-wing transformation observed in *Ubx*/+ animals (LEWIS 1954) suggests that segment identity is sensitive to changes in the level of Ubx^+ expression, the results presented here suggest that cellular identity depends upon the concentration of Ubx^+ product. In PS6, the extra doses of Ubx^+ and bxd^+ may alter the pattern of Ubx^+ expression. Anteriorly directed hairs, that in diploid animals appear in pA1(PS7), begin to appear in pT3(PS6). Furthermore, the pA1 row is denser in eight-dose animals than in wild-type animals, suggesting that additional cells have changed identity either because Ubx^+ product accumulates to a level normally

found in pA2 or because of the *de novo* synthesis of Ubx^+ product.

The pattern of Ubx^+ and bxd^+ expression with PS5 appears to change in response to the increased dosage of these gene functions if only one copy of the wild-type *Pc* gene is present. In animals deficient for *Pc*, the segments T1 through A8 resemble A8 (LEWIS 1978; DUNCAN and LEWIS 1982) and the *Ubx* pattern of expression within the central nervous system is altered (BEACHY *et al.* 1983; WEDEEN, HARDING and LEVINE 1986). These results suggest that the wild-type *Pc* function modulates the pattern of Ubx^+ and bxd^+ expression in each segment. To test the idea that the pattern of *Ubx* and *bxd* expression in PS5 could be altered to resemble that of PS6 by altering the doses of *Pc* relative to *Ubx* and *bxd*, I constructed animals carrying eight doses of the *Ubx* and *bxd* genes and either one or two copies of *Pc*. As stated above, larvae carrying eight copies of Ubx^+ and bxd^+ are indistinguishable from eight-dose animals that carry one copy of the wild-type *Pc* gene. In the eight-dose adults, the pattern of cells expressing *Ubx* and *bxd* in aT3 appears to be unaffected by changes in the dose of *Pc*. However, the pattern of cells expressing *Ubx* and *bxd* in pT2 is very sensitive to these changes and some of the PS5 cells that would normally develop into posterior wing now take on the identity of the PS6 cells destined to become haltere.

During embryogenesis, the parasegments are elaborated on the basis of complex interactions among a variety of segmentation genes. The complement of segmentation genes expressed varies among parasegments, and one might anticipate that this would result in different patterns of genetically distinct cell states within each parasegment. The variation in the response of the cells within PS5 to changes in the doses of Pc^+ and the Ubx^+ and bxd^+ functions may reflect such a mosaic character. The mosaicism may be based on the differential expression of activators and/or repressors essential for the development of the Ubx^+ and bxd^+ expression pattern within the parasegment or it could reflect relative differences in the availability of *Ubx cis*-regulatory regions among the cells of each parasegment.

Dosage interactions between the *ANT-C* and *Ubx/bxd*: The observed transformation of the third thoracic and eight abdominal segments of $BX\text{-}C^-$ larvae toward more anterior segmental identities (LEWIS 1978) suggests that *BX-C* genes might act to suppress the activity of the *Antp* gene in T3 through A8. The molecular analysis of animals deficient for the entire *BX-C* demonstrates that the *Antp* RNAs and proteins, which are normally expressed only in PS3 through PS5, are also expressed in each abdominal ganglion as well (HAFEN, LEVINE and GEHRING 1984; CARROLL *et al.* 1986). The observation that a mutation in the *BX-C* functions can affect the expression of *Antp* is consistent with the idea that *Antp* and *Ubx* interact to determine the identity of PS5, in which both *Antp* and *Ubx* are normally expressed (AKAM and MARTINEZ-ARIAS 1985).

AKAM and MARTINEZ-ARIAS (1985) have shown that cells in PS5 that express Ubx^+ do not express $Antp^+$ and those that do not express Ubx^+ express $Antp^+$. Combining this result with the observation that the T3 setal belt is intermediate between those of PS3 and PS6 in *Antp* deficient larvae (WAKIMOTO and KAUFMAN 1981; STRUHL 1983; MARTINEZ-ARIAS 1986), MARTINEZ-ARIAS (1986) proposed that early interactions between $Antp^+$ and Ubx^+ products might determine the wild-type expression of these loci in PS5. It may be that the observed cellular distribution of Ubx^+ and $Antp^+$ products reflects an underlying pattern of *Antp* and *Ubx* regulators, an interaction between the two genes or both. One copy of Pc^+ and eight copies of Ubx^+ and bxd^+ cause a few of the pT2 cells to take on a PS6 identity. The hemizygosity of *ANT-C* enhances this effect and many more of the pT2 cells of PS5 appear take on the identity of PS6 cells. The hemizygosity for *ANT-C* has no effect on the phenotype of $Pc^3/+$ heterozygotes that carry two, three or five doses of the *Ubx/bxd* region. One interpretation of this observation is that $Antp^+$, together with Pc^+, can suppress the activity of Ubx^+ in these cells. The results presented above support the hypothesis that the two genes interact and that the interaction is affected by Pc^+ product. While the composition of regulators in PS6 may allow Ubx^+ to inhibit *Antp* expression in the cells of this parasegment, it may be that the various regulators in the cells of PS5 and more anterior parasegments allow *Antp* to play a role in the inhibition of Ubx^+ expression. Alternatively, it may be that the decrease in Pc^+ function causes Ubx^+ to become active in cells in which it is normally inactive. Once expressed, it may repress $Antp^+$ in PS5 as it does in PS6. In animals with only one wild-type copy of the *ANT-C*, the effect could be greater because there would be fewer copies of the $Antp^+$ genes that must be repressed. Because deficiencies for the entire *ANT-C* were used in these studies, it may be that hemizygosity for other genes within the complex results in the enhanced wing to haltere transformation of $Pc^3/+$ heterozygotes that carry eight copies of the *Ubx/bxd* region. Although other *ANT-C* genes such as *Deformed* (*Dfd*), *Sex Combs Reduced* (*Scr*) and *fushi tarazu* (*ftz*) do not appear to be expressed in PS5 (MARTINEZ-ARIAS *et al.* 1987; WAKIMOTO and KAUFMAN 1981; HOWARD and INGHAM 1986), this possibility cannot be ruled out without data on animals deficient for *Antp* alone.

In conclusion, I have described the results of experiments designed to test the ability of multiple doses of

the *Ubx* and *bxd* functions to affect the transformation of PS5 toward PS6. These experiments demonstrate that additional copies of *Ubx* and *bxd* affected only a few PS5 structures, the fine hairs of the T3 setal belt, which are transformed toward the hook-like setae of the A1 setal belt. In the presence of eight copies of *Ubx/bxd* and one copy of *Pc*, some of the PS5 cells corresponding to pT2 take on the characteristics of PS6 cells corresponding to pT3. This effect is enhanced by reducing the dose of *ANT-C*. Because the dosage effects appear to affect only a subset of cells within each segment, these results support the hypothesis of PEIFER, KARSH and BENDER (1987) that segment identity is based on the pattern-specific expression of different homeotic genes. They also suggest that the levels of homeotic gene products expressed in a particular cell affects its identity.

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